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Methodological paper

Gender-specific association between *Apelin/APJ* gene polymorphisms and hypertension risk in Southeast China

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ABSTRACT

To explore the role of genetic factors in the pathogenesis of hypertension, our study investigated the gender-specific association between four polymorphisms in the *Apelin/APJ* gene and hypertension risk in southeastern Chinese population. All participants including 645 hypertensive patients and 362 normotensive controls were genotyped for 4 gene polymorphisms associated with hypertension susceptibility including *Apelin* (rs909656, rs5975126) and *APJ* (rs10501367, rs11544374). According to genotype analysis, for male subjects, the frequencies of genotypes ($P = 0.046$ and 0.046, respectively) of rs10501367 and rs11544374 revealed significant differences between the hypertension and control groups. Moreover, for female subjects, there was significant difference on the genotype distribution of rs11544374 between two groups ($P = 0.046$). The association of rs10501367 with hypertension was significant for males under additive models and recessive models, even after adjusting for age, BMI, fasting glucose and waistline. Besides, significant association was observed for rs11544374 in females under additive models. As for haplotype analysis, haplotype T-A (in order of rs10501367 and rs11544374) in *APJ* gene was marginally overrepresented in controls (17.9%) compared to patients with hypertension (11.6%) in males ($P = 0.003$). The mutation of polymorphism rs10501367 in *APJ* gene decreased risk of hypertension in Chinese males.

1. Introduction

Hypertension is estimated to cause 9.4 million deaths annually worldwide as well as hospitalization related to cardiovascular disease, kidney disease and stroke (Redon et al., 2016; Lim et al., 2012). It has also been identified as an independent increased risk factor for cardiovascular morbidity and mortality (Organization W H, 2009). Therefore, it is essential to investigate the factors affecting hypertension in order to minimize its damage.

Previous studies have shown that hypertension is a multifactorial, polygenetic disorder influenced by environmental factors and genetic variations (Qi et al., 2014). Among multiple factors, genetic elements are essential for blood pressure range in human essential hypertension (EH) (Fava et al., 2013). The family-based research estimates that approximately 30–60% of variance in blood pressure can be attributable to genetic variations (Agarwal et al., 2005). There are some reports on susceptibility candidate genes. An extensive body of research has demonstrated that apelin/APJ systems are involved in the pathogenesis of hypertension (Lu et al., 2016; Li et al., 2015).

Recent studies have found that apelin, which is a 36-amino acid peptide extracted from bovine stomach (Kazuhiko et al., 2012), can regulate blood pressure through its APJ receptor by facilitating peripheral and coronary vasodilatation, reducing the arterial blood pressure, diminishing cardiac preload and afterload, and by enhancing cardiac output (Barnes et al., 2013; Cao et al., 2015; Wu et al., 2014). Besides, accumulating evidence has confirmed that *Apelin/APJ* gene polymorphisms are associated with hypertension. A pilot exploratory case–control study has found haplotype-based associations of four well

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; ApoA, apolipoprotein A; ApoB, Apolipoprotein B; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; UA, urea acid; Cr, creatinine; 95% CI, 95% confidence interval; OR, odds ratio

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characterized polymorphisms (T-1860C, rs3761581, rs7119375 and rs10501367) in apelin–AGTRL1 system with hypertension: haplotypes C–C–G–G (in order of T-1860C, rs3761581, rs7119375 and rs10501367) and T–A–A–A have shown to be associated with significantly higher risk of developing hypertension after adjusting for several factors, including age, onset age, body mass index and waist-to-hip ratio, whereas haplotype C–C–A–A has shown to play a protective role in hypertension (Niu et al., 2010). Another replication study has further demonstrated that the mutation of promoter polymorphism rs7119375 in *APJ* gene is associated with elevated systolic blood pressure, and that rs7119375 of the *APJ* gene confers susceptibility to hypertension in women (Li et al., 2016). Existing studies have identified increasing number of new loci. In 2014, Liu et al. have observed APJ (rs7119675 and rs11544374) are significantly associated with hypertension among all genetics models, and the association was independent of confounding factors including age, gender and BMI (Liu et al., 2014). Considering these observations, it is plausible to suggest that *Apelin/APJ* genes are responsible for the occurrence and development of hypertension.

To shed more light on this issue, we decided to make a thorough inquiry of the gender-specific association between 4 well-defined polymorphisms in apelin/APJ system and both blood pressure changes, as well as with hypertension risk in southeast China.

2. Materials and methods

2.1. Participant recruitment

This was a hospital-based case-control study including 1007 Han Chinese recruited from the First Affiliated Hospital of Fujian Medical University, and all underwent a complete medical history and physical examination during the same time period. 362 healthy control subjects with systolic and diastolic blood pressures (DBP) < 140 and < 90 mm Hg who were not taking any antihypertensive medications, and 645 patients with conditions which included hypertension were admitted into the study. According to the criterion of China's prevention and care guidelines for hypertension, the hypertension diagnosis was defined as mean systolic blood pressures (SBP) ≥ 140 mm Hg or DBP ≥ 90 mm Hg. Also, the blood pressure was measured three times using a calibrated mercury sphygmomanometer by certified examiners with at least 10 min interval and taken the average of three readings, or before the use of at least 1 class of antihypertensive drugs. Patients were excluded from the study for any of the following conditions: severe heart diseases (e.g. myocardial infarction, congenital heart disease, serious arrhythmia, valvulopathy or other atherosclerotic lesions), cancer, severe anemia, severe metabolic disease, severe psychiatric disturbance, peripheral vascular disease, renal failure, infectious diseases. All participants signed an informed written consent prior to the study. The project was approved by the Institute's Human Research and Ethics Committees and was conducted according to the Declaration of Helsinki Principles.

2.2. General clinical characteristics

All participants were surveyed via standardized questionnaire that included information on gender, age, weight, height, waist circumference, smoking history and alcohol drinking habits. BMI was calculated as weight (kg)/height^2 (m)^2. Approximately 5 ml of fasting whole blood samples from all subjects were collected in the supine position after an overnight fasting. Fasting blood glucose was measured by automatic analysis instrument (Beckman CX-7 Biochemical Autoanalyzer; Brea, CA). Serum concentrations, including total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C), creatinine (Cr), uric acid (UA) were measured with a type 7600–020 automated analyzer (Hitachi, Tokyo, Japan).

2.3. Genotyping

Genomic DNA from all subjects was extracted from the peripheral blood, which was anticoagulated in EDTA and stored at $-20\,^{\circ}C$ until required for batch genotyping, using DNA isolation kits supplied by Xiamen Taijing Biological Technology Co. Ltd. Four examined single nucleotide polymorphisms (rs909656, rs5975126, rs10501367, rs11544374) were determined by PCR-LDR (polymerase chain reaction-ligase detection reactions) method using 5′ TaqMan genotyping assays applied by Shanghai ShenGong Biological Engineering Service Co. Ltd. on a Light Cycler 480 Instrument (Roche Diagnostics Ltd., Basel, Switzerland) according to the manufacturer's instructions. The *Apelin/APJ* genes' Taqman probes and primers were synthesized by Shanghai Generay Biotech Co. Ltd. PCR amplification was carried out in a PTC-200 MJ Research Peltier Thermal Cycler (Bio-Rad Lab, Massachusetts, USA). The detailed procedure of four examined polymorphisms genotypes identification was performed as previously described (Zeng et al., 2014).

2.4. Statistical analysis

Demographic and clinical variables from all participants were analyzed using SPSS v.21.0. As the gene encoding apelin is mapped on the X chromosome (Lee et al., 2000), the genotype-hypertension analysis was conducted in males and females separately. For the calculation and comparison of clinical statistical measurement data, homogeneity of variance and normality test were carried out. Data were reported as means ± standard deviation (SD). Normally distributed numeric variables between two groups were compared using the Student's *t*-test and non-parametric test was used when normality test was not satisfied, while multiple group means were analyzed using one-way ANOVA. The $\chi 2$ test was used for genotype/allele frequencies. Binary logistic regression was applied to the risk prediction for hypertension under additive models, dominant models and recessive models, and the confounding factors including age, BMI, fasting glucose and waist circumference were adjusted. The values of effect-size were represented by odds ratio (OR) and its 95% confidence interval (95% CI). Hardy-Weinberg equilibrium and haplotype analysis were calculated using SHESIS software. The analysis results were also processed using online calculator for FDR correction (https://www.sdmproject.com/utilities/?show=FDR). Two-tailed $P < 0.05$ was considered as statistically significant. The power of detecting this significance was over 95%.

3. Results

3.1. General clinical characteristics

Table 1 presents the comparison in baseline characteristics including anthropometric index and clinical biomarkers between patients with hypertension (378males, 267 females) and controls (203 males, 159 females). No statistical differences were observed in ApoB, TC and L-DLC between patients and controls in both genders after FDR correction ($P > 0.05$). In contrast, male and female patients had significantly higher levels of age, waist circumference, BMI, fasting glucose, SBP, DBP, pulse pressure (PP), TG, UA and Cr, but significantly lower levels of H-DLC than controls, respectively ($P < 0.05$). Apart from this, higher levels of ApoA was exclusively found in male controls compared with male hypertension patients ($P < 0.001$).

3.2. Single-locus analysis

Because *Apelin* gene is assigned to X chromosome, Hardy-Weinberg equilibrium test of rs909656 and rs5975126 were only performed in females, while the equilibrium for the *APJ* gene was assessed in both genders. Based on the |D'| values, the linkage disequilibrium analysis showed that rs909656 and rs5975126 were not linked (Fig. 1).